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TITLE: Characterization of the Discoidin Domain Receptor 2 Kinase as a Novel Therapeutic Target for Squamous Cell Lung Cancer

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14. ABSTRACT

This program has undertaken the study of the discoidin domain 2 (DDR2) tyrosine kinase as a potential therapeutic target in squamous cell lung cancer. We identified mutations in the DDR2 gene in a cohort of 290 individuals with this type of lung cancer and showed that these mutations are oncogenic when expressed in cell lines. Importantly, we showed that DDR2-driven cellular transformation sensitizes cancer cells to treatment with tyrosine kinase inhibitors which target DDR2, namely dasatinib. We have generated cellular and animals models to study DDR2, have studied the mechanisms by which DDR2 transforms cells and have worked to develop more selective inhibitors of DDR2 as well as identify mechanisms of cellular resistance to dasatinib therapy. This work has led to a publication in Cancer Discovery and has been presented at several scientific meetings including AACR and IASLC. Dasatinib is now being studied in the clinic as a DDR2 inhibitor in a national phase II trial for patients with squamous cell lung cancer, a disease for which no targeted agents are approved at this time.

15. SUBJECT TERMS
squamous lung cancer, targeted therapeutics, tyrosine kinases

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Introduction

Lung cancer remains the leading cause of cancer-related mortality in the United States with nearly 170,000 deaths per year. While substantial progress has been made over the past decade in genomic characterization and development of targeted therapeutics for lung adenocarcinoma, the most common type of lung cancer, little progress has been made in the second most common type of lung cancer, squamous cell carcinoma. This has led to a disparity in treatment in which individuals with lung adenocarcinoma are offered personalized therapies based on the genotype of their tumors while individuals with squamous cell carcinoma are treated with the same chemotherapy drugs as a decade ago. In lung adenocarcinoma personalized treatment approaches have led to improved survival for patients with advanced disease and advances of this nature are desperately needed in lung squamous cell carcinoma.

This research program began with our observation that the discoidin domain receptor 2 (DDR2) kinase is a possible therapeutic target in squamous lung cancer. We identified *DDR2* mutations in 11/290 squamous cell lung cancer samples and demonstrated that several of these mutations can drive cellular transformation as measured by anchorage-independent growth of NIH-3T3 cells and interleukin-3 (IL-3) independent proliferation in Ba/F3 cells. We showed that two lung cancer cell lines harboring *DDR2* mutations are sensitive to a number of tyrosine kinase inhibitors, most notably the FDA-approved tyrosine kinase inhibitor dasatinib, which potently inhibits DDR2. With the support of the DOD we have performed a series of experiments to establish *DDR2* mutations as likely therapeutic targets and have studied how these mutations drive cellular transformation. Further, as a national trial has now opened to study dasatinib as a DDR2 inhibitor in patients, we have studied potential mechanisms of acquired resistance to dasatinib in the setting of *DDR2* mutations and have worked to identify more selective therapeutic strategies for individuals with *DDR2* mutations.

Body

Aim 1: Determine whether DDR2 is required for survival of squamous lung cancer cell lines with DDR2 mutations.

shRNA analysis of DDR2 essentiality. As reported in the Year 1 progress report and in Hammerman et al., 2011, we have analyzed two lung cancer cell lines with *DDR2* mutations for *DDR2* essentiality by shRNA knockdown. These two cell lines, NCI-H2286 and HCC-366, were sensitive to *DDR2* knockdown as measured by a decrease in cell proliferation using two independent shRNAs. This phenotype was rescued by ectopic expression of a *DDR2* cDNA which did not contain the shRNA targeting sequence. shRNAs targeting *DDR2* were not found to decrease proliferation in a dasatinib sensitive cell line (NCI-H1703) and a dasatinib insensitive cell line (A549), neither of which were found to harbor *DDR2* mutations. *DDR2* knockdown was associated with a decrease in both cell proliferation and in markers of DDR2 signaling including p-Src and p-STAT3.

This analysis has now been extended in Year 2 to a larger panel of lung cancer cell lines as part of Project Achilles (PI Dr. William Hahn) who has assessed 200 cell lines for shRNA sensitivity using multiple shRNAs corresponding to over 15,000 genes. Preliminary analysis of this dataset has not identified additional squamous lung cancer cell lines which display sensitivity to shRNAs targeting *DDR2*, though none of these cell

lines harbors a *DDR2* mutation, so this result is not surprising. Attempts at generating inducible expression of shRNAs targeting *DDR2* have unfortunately not been successful to the point at which we were comfortable performing *in vivo* validation, though efforts continue to develop the necessary reagents for these experiments.

Aim 2: Explore the use of the tyrosine kinase inhibitors imatinib, nilotinib and dasatinib as anticancer therapies via inhibition of DDR2.

Small Molecule Inhibitors of DDR2. As reported in the Year 1 report and in Hammerman et al., we profiled *DDR2* mutated lung cancer cell lines for sensitivity to over 20 tyrosine kinase inhibitors. The most potent inhibitors of *DDR2*-mutated cell lines were dasatinib, an FDA-approved agent, and ponatinib, an agent currently in late-phase clinical trials for the treatment of patients with imatinib-resistant chronic myelogenous leukemia. These agents both resulted in apoptosis in *DDR2*-mutated cell lines and resulted in decreased levels of p-DDR2 and downstream signaling proteins. These studies were extended in xenograft models and additional sequencing studies did not identify additional squamous cell lung cancer cell lines with *DDR2* mutations.

In Year 2 we have continued to study pharmacologic inhibition of DDR2 in NCI-H2286 and HCC-366, two cell lines we established as *DDR2* mutated and *DDR2*-dependent. Given that dasatinib has moved forward clinically in trials as a DDR2 inhibitor, we have begun to study potential mechanisms of acquired resistance to dasatinib since acquired resistance universally occurs in patients with lung cancer who are treated with targeted kinase inhibitors. To study acquired resistance to dasatinib we generated NCI-H2286 and HCC-366 cell lines which were able to sustain growth in concentrations of dasatinib 10 times their natural IC_{50}s (1.5 micromolar). We extracted genomic DNA from these cell lines and performed hybrid-capture based sequencing targeting over 700 cancer related genes using the Oncopanel platform. Interestingly, we identified only one genomic alteration in the resistant HCC-366 population as compared to the parental cell line—a second-site mutation in *DDR2* T654I (Figure 1). This mutation had previously been reported by our group and others to be a gatekeeper mutation which renders DDR2 dasatinib-insensitive. In NCI-H2286 we identified a number of mutations in genes other than *DDR2* and no-second site *DDR2* mutations in analysis of both a pooled culture of resistant cells and in independent clones generated from this pool (Figure 2). We have been analyzing these alterations for their ability to drive dasatinib resistance with loss of *NF1* being the most promising to date (Figure 3). Additional mutated alleles are being characterized at this time both alone and in combination with others to examine which events are capable of driving dasatinib resistance.

Clinical trials of dasatinib have frequently reported substantial toxicity associated with drug administration. In fact, the patient reported in Hammerman et al. who harbored a *DDR2* mutation and who responded to combination therapy with dasatinib and erlotinib discontinued the trial due to toxicity and not lack of efficacy. In our own clinical trial of dasatinib for patients with squamous cell lung cancer we have observed substantial fatigue as well as pleural effusions in two patients, a potentially very serious complication for individuals with lung cancer. Given these observations we have begun work to develop more selective inhibitors of DDR2 with Dr. Nathanael Gray's laboratory and have identified lead compounds which appear to have low nanomolar affinity for DDR2. Interestingly, as will be discussed in the report under Aim 3, while these inhibitors are not very active as single agents they display inhibition of DDR2-dependent cell lines when combined with Src inhibitors, suggesting that this may be an attractive therapeutic

strategy.

Aim 3: Characterize the oncogenic transformation mediated by mutated forms of the discoidin domain receptor 2 (DDR2) kinase and to elucidate the mechanism by which mutated DDR2 functions as an oncogene.

DDR2 overexpression studies. As detailed in the Year 1 report and in Hammerman et al., we developed transformation systems (NIH-3T3, AALE and Ba/F3) to study the oncogenic potential of *DDR2* mutations. While only a minority of the mutants drove transformation in the NIH-3T3 cells, transformation was robust in the Ba/F3 system, perhaps due to differences in the cellular background. *DDR2* mutations did not drive transformation in AALE cells. Studies of the Ba/F3 and NIH-3T3 lines showed that *DDR2* driven transformation could be blocked by several tyrosine kinase inhibitors and most notably by dasatinib.

Generation of DDR2 transgenic mice. As reported in the Year 1 report we generated transgenic animals expressing both *DDR2* L63V and I638F given that these mutants displayed the most impressive transformation in NIH-3T3 and Ba/F3 cells. After one year of observation we did not observe tumor formation with the exception of two of 40 animals in which nodules were observed but *DDR2* expression was not confirmed by IHC, suggesting the tumors were not *DDR2* driven. Attempts were then made to accelerate tumor formation by crossing these animals to cancer-prone strains. As reported last year, *DDR2* mutation did accelerate *EGFR* driven tumorigenesis, though the clinical relevance of this model is questionable given the recent report by the TCGA Network which suggests that *EGFR* mutations are very rare in squamous cell lung cancers. Crossing of the *DDR2* transgenic animals to a *TP53* null background resulted in very rare birth of animals representing the compound genotype, suggesting a developmental role for wild-type *DDR2* in this context. We have recently just generated compound *DDR2* transgenic/*PTEN* null animals and are observing these animals for tumor formation at this time.

Elucidating the mechanism of transformation mediated by mutated DDR2. As reported previously, we observed increased p-STAT3, p-STAT5 and p-Src in cancer cell lines and isogenic models driven by mutated *DDR2*. Consistent with this observation, another groups reported that DDR2 and Src bind one another and that Src is required for DDR2 activity, work we have reproduced in our lab. These observations led us to probe the relationship among DDR2 and Src in more detail with a focus on combination inhibitor studies given the toxicity of dasatinib, a dual DDR2 and Src inhibitor. We observed that selective inhibitors of DDR2, which had little effect on DDR2 phosphorylation or viability of *DDR2* mutated cell lines, demonstrated synergy with AZD0530, a clinically relevant selective Src inhibitor (Figures 4 and 5). This was true for both ALW-II-49-7, a drug developed at Harvard Medical School as a DDR inhibitor as well as nilotinib and ponatinib, two tyrosine kinase inhibitors which target DDR2 and have a favorable toxicity profile as compared to dasatinib. These results suggest that more selective inhibitors of DDR2 plus Src inhibitors may be active against *DDR2* mutated tumors and could avoid the toxicity of dasatinib. These results are preliminary at this time and are the focus of a specific aim of my recently funded K08 award. Given the focus on dual targeting of Src and DDR2 we have not performed an unbiased screen to identify other DDR2 interacting proteins.

Key Research Accomplishments

- Identification of *DDR2* mutations in 3-4% of squamous cell lung cancers and association of *DDR2* mutation with response to specific tyrosine kinase inhibitors (Year 1 report and Hammerman et al.)
- Broad profiling of lung cancer cell lines for *DDR2* essentiality
- Generation of dasatinib resistant *DDR2* mutated cell lines and identification of genomic alterations associated with dasatinib resistance, notably second-site mutations in *DDR2* and loss of *NF1*
- Generation of more selective inhibitors of DDR2
- Identification of dual DDR2 and Src inhibition as a therapeutic strategy for tumors harboring *DDR2* mutations
- Generation of *DDR2* transgenic animals

Reportable Outcomes

- First author publication describing *DDR2* mutations and association with response to dasatinib in *Cancer Discovery*
- Oral presentations at AACR in 2011 and 2012; 2011 given by laboratory mentor Dr. Matthew Meyerson
- Award for best abstract at Harvard Lung Cancer SPORE Retreat, 2011
- Plenary talk at World Lung Cancer Congress, 2011
- Invited presentation at the 12th Annual Targeted Therapies of Lung Cancer Meeting, 2012
- Invited presentation at the 2012 Chicago Multidisciplinary Symposium in Thoracic Oncology
- Invited presentation at the 2012 International Thoracic Oncology Congress, Dresden, Germany
- Successful NCI K08 application for additional work on *DDR2* and other therapeutic targets in squamous cell lung cancer
- IRB approval of national trial of dasatinib for individuals with squamous cell lung cancer and *DDR2* mutations; study opened locally in November of 2011 and nationally in 2012
- Selection as leader of writing and analysis committee for The Cancer Genome Atlas project in squamous cell lung cancer; publication in *Nature* in September 2011
- Tenure-track faculty position offers as Assistant Professor at Vanderbilt University, MD Anderson Cancer Center, Dana-Farber Cancer Institute and Ontario Cancer Institute
- Selection for Grants Review Committee, National Lung Cancer Partnership
- Reviewer for *Clinical Cancer Research* and *Journal of Thoracic Oncology*

Conclusions

The work presented in this report has identified mutations in *DDR2* as likely therapeutic targets in squamous cell lung cancer, a common type of lung cancer for which no therapeutic targets are approved. Activities in Year 1 included the documentation of the prevalence of *DDR2* mutations and association with sensitivity to several tyrosine klnase inhibitors, most notably dasatinib. In Year 2 work has focused on describing mechanisms of acquired resistance to dasatinib, developing more selective DDR2 inhibitors given toxicity associated with dasatinib and the study of signaling downstream

of DDR2 to identify additional targeted approaches for *DDR2* mutated cancers. These studies have been accompanied by an investigator-initiated phase II trial of dasatinib in squamous cell lung cancers which is accruing patients at this time, both at my current institution and at other cancer centers across the country. This fellowship has been instrumental in supporting my development as a physician-scientist and was critical in allowing me to develop the necessary data to secure a K08 award and to prepare for a transition to a fully independent position.

Personnel List

Peter Hammerman, MD PhD
Brittany Woods, BA (2011-2012), now currently a PhD student at Memorial Sloan Kettering Cancer Center
Ellen Beauchamp, BS

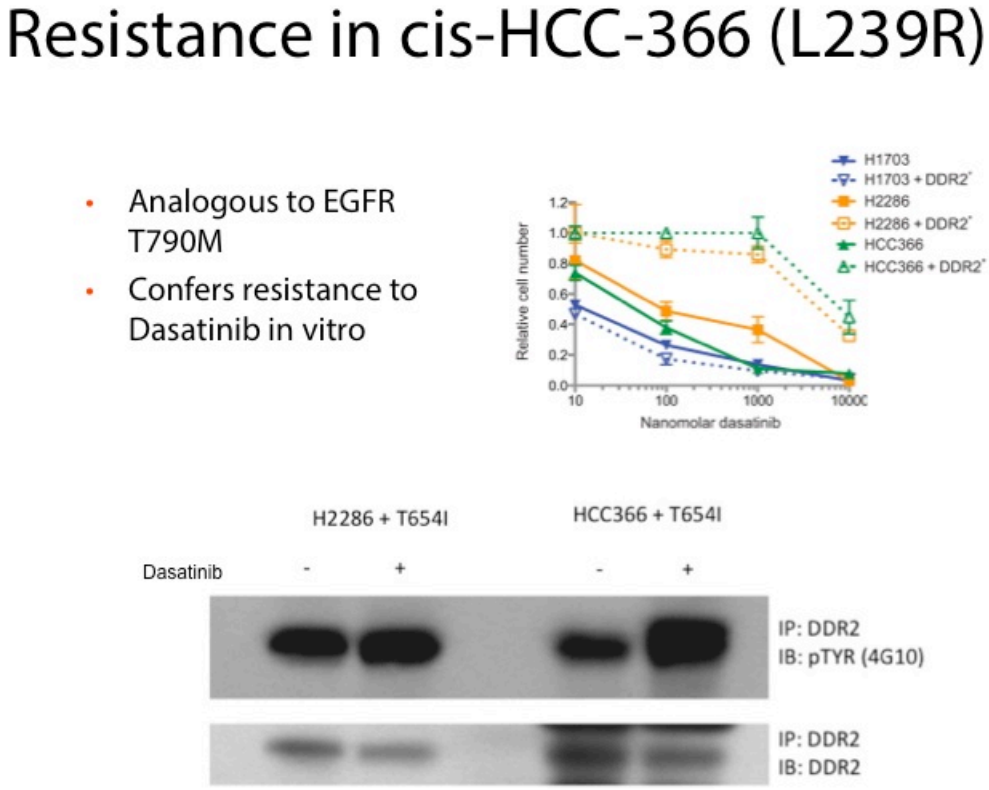
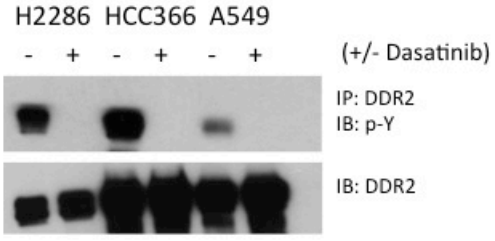


Figure 1: A second site mutation in *DDR2* (T654I) drives resistance to dasatinib. Top: Proliferation of three dasatinib sensitive cell lines (H1703-*DDR2WT)* and H2286 and HCC366 (both *DDR2* mutated) measured by Cell-Titer-Glo after six days of dasatinib treatment at the indicated concentrations. In the cases where *DDR2'* is noted the cell lines are ectopically expressing *DDR2* T654I. This transgene shifts the IC_{50} curves for both *DDR2* mutated cell lines. Bottom: Immunoprecipitation of DDR2 from cell lines expressing *DDR2* T654I followed by 4G10 western blot for phosphotyrosine. In contrast to the data presented in the Year 1 report, no effect on DDR2 phosphorylation is noted when the T654I mutation is expressed. For reference the blot presented last year is shown below for endogenous DDR2.



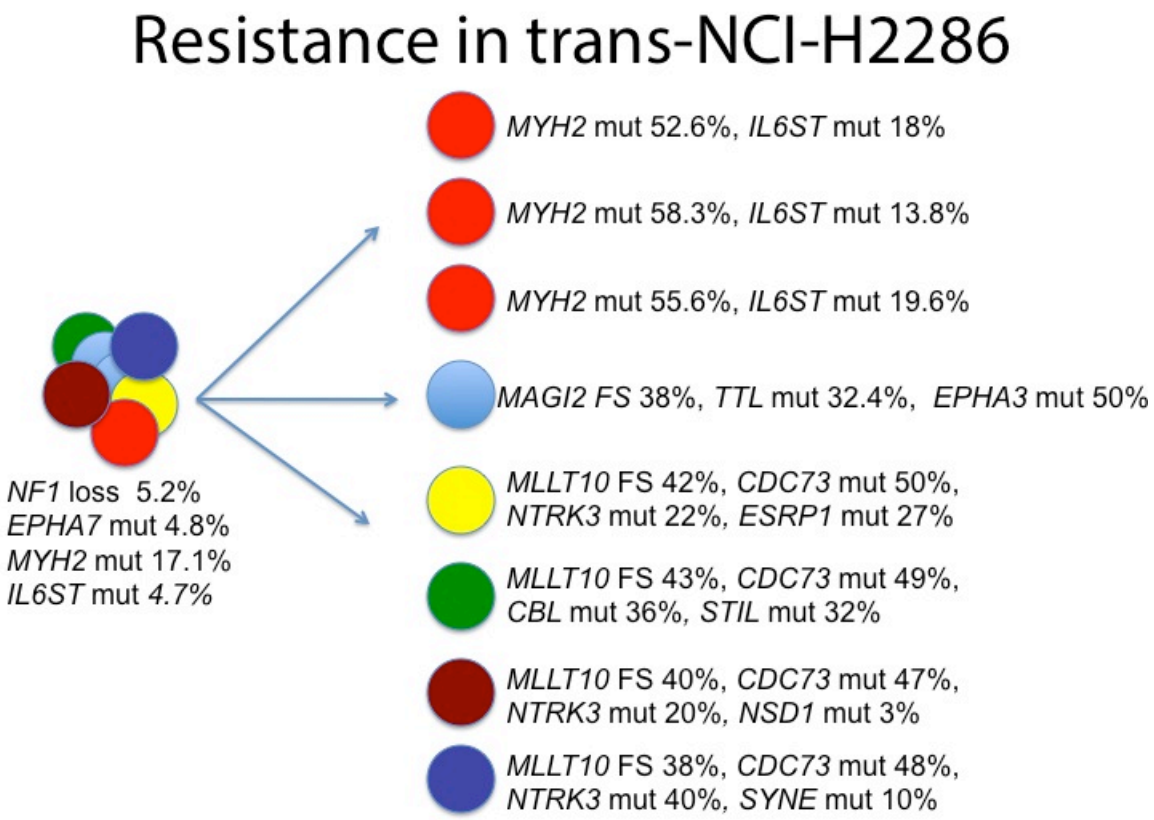


Figure 2: Sequencing of 700 cancer related genes in dasatinib-resistant NCI-H2286 cells. Results from the initial pool are shown on the left and individual clones on the right. Percentages indicate the allelic fraction of identified alterations.

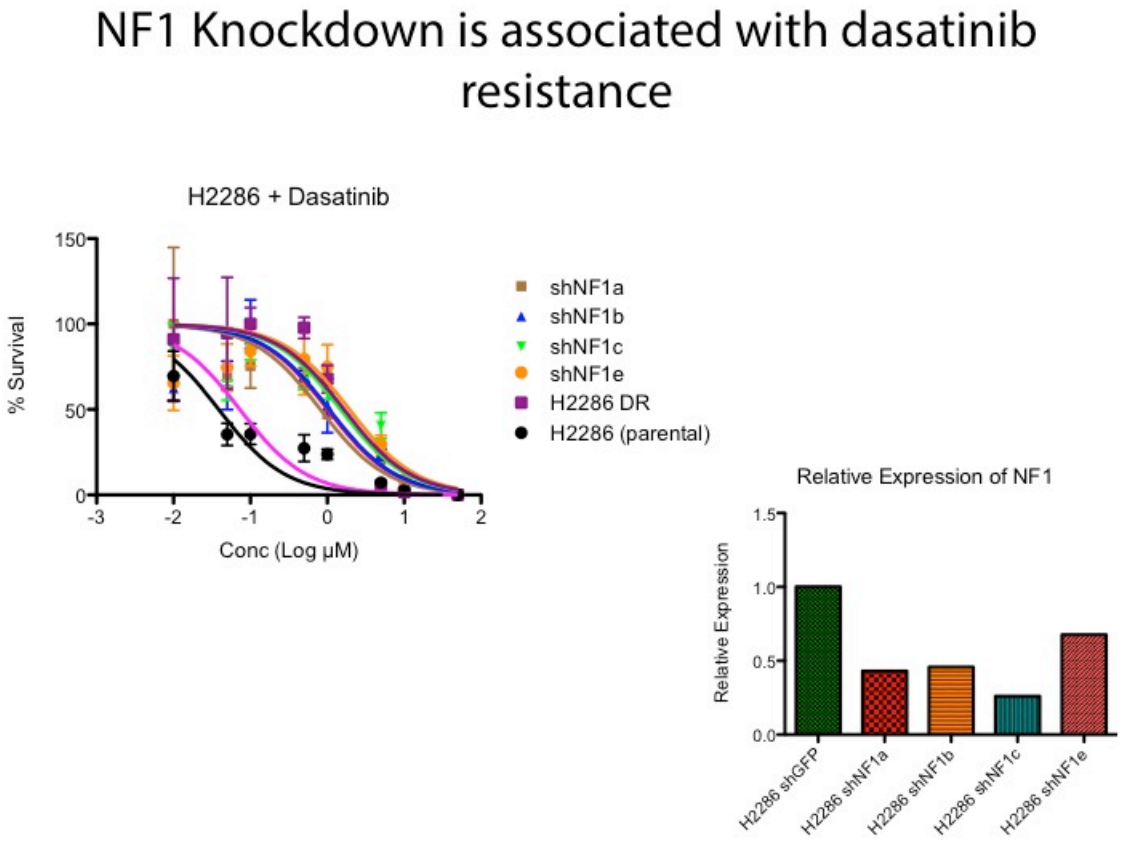
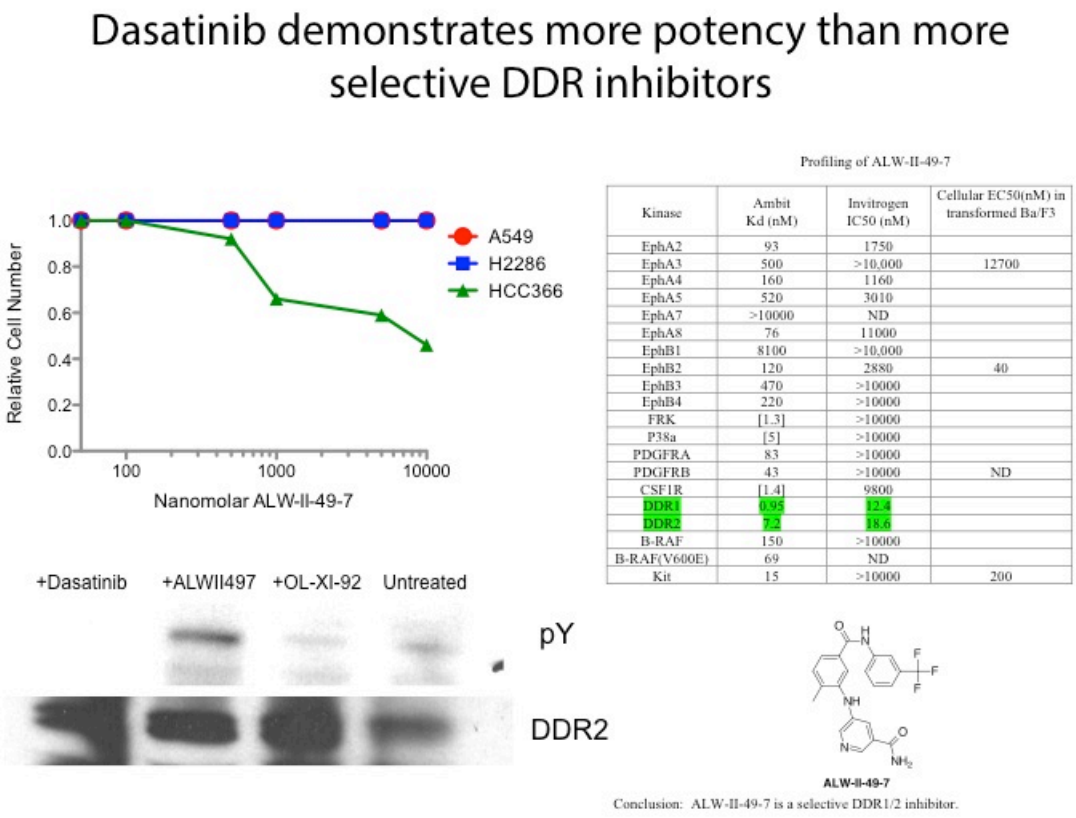


Figure 3: *NF1* knock-down leads to dasatinib resistance in NCI-H2286 cells. Top left: Proliferation measured of *DDR2* mutated NCI-H2286 cells (parental) and dasatinib resistant (DR) NCI-H2286 cells after six days of dasatinib treatment. For the lines labeled shNF1 NCI-H2286 cells were infecting with lentivirus expressing one of four shRNAs targeting *NF1*. Bottom right: Real-time PCR measurement of *NF1* knockdown as compared to shGFP in NCI-H2286 cells.



Kinase	Ambit Kd (nM)	Invitrogen IC50 (nM)	Cellular EC50(nM) in transformed Ba/F3
EphA2	93	1750	
EphA3	500	>10,000	12700
EphA4	160	1160	
EphA5	520	3010	
EphA7	>10000	ND	
EphA8	76	11000	
EphB1	8100	>10,000	
EphB2	120	2880	40
EphB3	470	>10000	
EphB4	220	>10000	
FRK	[1.3]	>10000	
P38a	[5]	>10000	
PDGFRA	83	>10000	
PDGFRB	43	>10000	ND
CSF1R	[1.4]	9800	
DDR1	0.95	12.4	
DDR2	7.2	18.6	
B-RAF	150	>10000	
B-RAF(V600E)	69	ND	
Kit	15	>10000	200

Figure 4: Development of ALW-II-49-7, a selective DDR kinase inhibitor. Top right: Profiling of ALW-II-49-7 *in vitro* shows potential activity against DDR2. Top left: ALW-II-49-7 demonstrates little to no killing of two *DDR2* mutated cell lines or A549. Bottom left: ALW-II-49-7 does not lead to DDR2 dephosphorylation in NCI-H2286 cells in contrast to dasatinib. OL-XL-92 is a compound with related structure to ALW-II-49-7 but without the same degree of DDR2 selectivity.

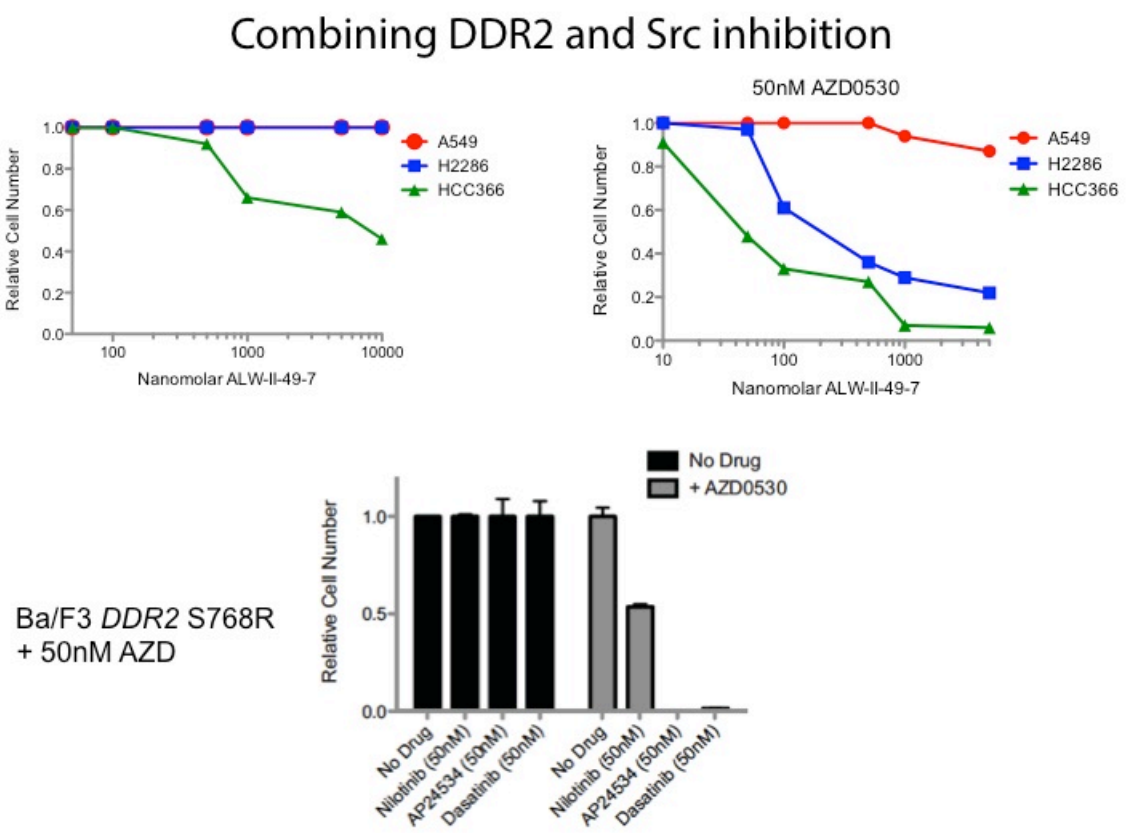


Figure 5: Combination treatment with more selective DDR2 inhibitors and the selective Src inhibitor AZD0530. Top left: Reproduced from above, showing little effect of ALW-II-49-7 on *DDR2* mutated cell lines. Top right: 50nM ALW-II-49-7 plus the selective Src inhibitor AZD0530 leads to reduced proliferation of *DDR2* mutated cell lines. As reported in Hammerman et al AZD0530 has minimal activity alone in these lines. Bottom: Ba/F3 cells expressing *DDR2* S768R, the mutation described in a patient with the clinical response to dasatinib, are sensitive to low concentrations (50nM) of the more selective DDR2 inhibitors nilotinib and AP24534 plus 50 nM AZD0530.

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RESEARCH ARTICLE

Mutations in the *DDR2* Kinase Gene Identify a Novel Therapeutic Target in Squamous Cell Lung Cancer

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ABSTRACT

Although genomically targeted therapies have improved outcomes for patients with lung adenocarcinoma, little is known about the genomic alterations that drive squamous cell cancer (SCC) of the lung. Sanger sequencing of the tyrosine kinome identified mutations in the *DDR2* kinase gene in 3.8% of lung SCCs and cell lines. Lung SCC cell lines harboring *DDR2* mutations were selectively killed by knockdown of DDR2 by RNA interference or by treatment with the multitargeted kinase inhibitor dasatinib. Tumors established from a *DDR2* mutant cell line were sensitive to dasatinib in xenograft models. Expression of mutated *DDR2* led to cellular transformation that was blocked by dasatinib. A patient with lung SCC that responded to dasatinib and erlotinib treatment harbored a *DDR2* kinase domain mutation. These data suggest that gain-of-function mutations in *DDR2* are important oncogenic events and are amenable to therapy with dasatinib. Because dasatinib is already approved for use, these findings could be used to rapidly generate clinical trials.

SIGNIFICANCE: *DDR2* mutations are present in 4% of lung SCCs, and *DDR2* mutations are associated with sensitivity to dasatinib. These findings provide a rationale for designing clinical trials with the FDA-approved drug dasatinib in patients with lung SCCs. *Cancer Discovery; 1(1). ©2011 AACR.*

INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality in the United States, with >157,000 deaths projected in 2010 (1). The more common type of lung cancer, non–small-cell lung cancer (NSCLC), accounts for 85% of cases and carries a grim prognosis; 70% of patients present with advanced and often incurable disease at the time of diagnosis (2).

Despite these statistics, a great deal of progress has been made in the targeted treatment of patients with NSCLC, largely owing to the development of small-molecule inhibitors of the epidermal growth factor receptor (EGFR) tyrosine kinase (3–5). Patients who respond to EGFR kinase inhibitors are much more likely to have the adenocarcinoma subtype of NSCLC (6). Patients with the other principal subtype of NSCLC, lung squamous cell cancer (SCC), very rarely respond to these agents; few advances have been made in the treatment of this type of lung cancer, which accounts for 25% of NSCLC. In addition to EGFR, several other promising therapeutic targets have been identified in the laboratory, including EML4-ALK, KRAS, and MET; drugs directed against these proteins are being tested in clinical trials (7–10). However, it appears that these targets are likely limited to adenocarcinomas as well. A recent report has suggested that targeting *FGFR1* amplifications in SCC of the lung may be a promising therapeutic strategy, although fibroblast growth factor receptor inhibitors are not currently in clinical use for the treatment of lung cancer (11). Given the burden of disease from lung SCC, we sought to identify new therapeutic targets by examining the tyrosine kinome of lung SCCs for novel mutated kinases.

In this article, we report the identification of novel somatic mutations in the discoidin domain receptor 2 (*DDR2*) tyrosine kinase gene at a frequency of 3.8% ($n = 11$) in a sample set of 290 lung SCC samples. DDR2, a receptor tyrosine kinase that binds collagen as its endogenous ligand, has been previously shown to promote cell migration, proliferation, and survival when activated by ligand binding and phosphorylation (12–18). *DDR1* and *DDR2* mutations have been noted in several cancer specimens, including 4 *DDR1* mutations (W385C, A496S, F866Y, and F824W) and 2 *DDR2* mutations in lung cancer (R105S and N456S); these reports, however, have not been confirmed

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Note: Supplementary data for this article are available at Cancer Discovery Online (http://www.aacrjournals.org).

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in independent samples, and functional characterization of the mutations has not been reported (19–21). We show that *DDR2* mutation status is associated with sensitivity to the tyrosine kinase inhibitor dasatinib or to short hairpin RNA (shRNA)–mediated depletion of DDR2. We also show that *DDR2* mutations are oncogenic and that their ability to transform cells can be blocked by treatment with dasatinib or with a combination of tyrosine kinase inhibitors. Furthermore, we report a *DDR2* kinase domain mutation in a clinical trial subject with SCC of the lung who had a radiographic response to combination therapy with erlotinib and dasatinib and who did not have an *EGFR* mutation. Together, these data suggest that *DDR2* may be an important therapeutic target in SCCs.

RESULTS

DDR2 Is Mutated in Lung SCC

We performed Sanger sequencing of 201 genes, including the entire tyrosine kinome, in an initial set of 20 primary lung SCC samples and matched normal controls; we identified somatic missense mutations in 25 genes in our discovery sample set, including 6 in tyrosine kinase genes (Fig. 1A). Recurrent somatic mutations were identified in *TP53* ($n = 8$) and in the tyrosine kinase genes: *DDR2* ($n = 2$) and kinase insert domain receptor (*KDR*; $n = 2$; Fig. 1A). Subsequent sequencing of 6 of the mutated tyrosine kinase genes (*DDR2*, *FGFR2, NTRK2, JAK2, FLT3,* and *CDK8)*, selected on the basis of being possible therapeutic targets, in a secondary screen of 48 lung SCC samples, including 13 cell lines, revealed 4 additional *DDR2* mutations (Fig. 1A) as well as 3 *FLT3* mutations, 2 *NTRK2* and *JAK2* mutations, and 1 mutation in each of *FGFR2* and *CDK8*.

Given that *DDR2* was the most frequently mutated gene in the primary and secondary screens, we sequenced *DDR2* in a validation cohort of 222 primary lung SCC samples, which yielded an additional 5 samples with mutation, resulting in an overall frequency of 3.8% ($n = 11$) in 290 total samples and an overall frequency of 3.2% in primary lung SCC samples when cell lines were excluded ($n = 9$ of 277; Fig. 1A). Mutations were found both in the kinase domain and in other regions of the protein sequence, and 2 mutations were identified at G774 (Fig. 1B). The L239R and I638F mutations were identified in the HCC-366 and NCI-H2286 SCC cell lines, respectively, and the remainder of the mutations were found in primary SCC samples. The majority of the mutations resided in regions of high degrees of amino acid conservation, compared with the murine, zebrafish, and *Caenorhabditis elegans* homologs of DDR2 (Supplementary Fig. S1). Additional genomic analysis of previously reported copy number and gene expression datasets did not reveal any evidence of *DDR2* overexpression in SCCs, compared with normal lung or lung adenocarcinoma, nor did we identify copy number alterations in *DDR2* (data not shown; refs. 19, 22–24). A query of the limited clinical information accompanying the sequenced samples did not identify any significant correlation of *DDR2* mutation status with age, sex, or smoking status of the patients.

DDR2 Mutant Cell Lines Are Selectively Sensitive to Tyrosine Kinase Inhibitors

To assess whether targeting DDR2 might be a promising therapeutic strategy in lung SCC, we analyzed several tyrosine kinase inhibitors reported to inhibit DDR2, including imatinib and dasatinib, drugs that are FDA approved for clinical use in targeting BCR-Abl in chronic myelogenous leukemia and acute lymphoblastic leukemia, c-KIT in gastrointestinal stromal tumors, and platelet-derived growth factor receptor in chronic myelomonocytic leukemia (21, 25–28). Fluorescence resonance energy transfer (FRET) measurements provided *in vitro* dissociation rate constant (K_d) values of dasatinib (5.4 nM) and imatinib (71.6 nM) for recombinant DDR2 (Supplementary Table S1)

Dasatinib showed particular efficacy against SCC cell lines bearing *DDR2* mutations because it inhibited proliferation of the *DDR2* mutant NCI-H2286 and HCC-366 cells with calculated 50% inhibitory concentrations (IC_{50}) of 139 and 140 nM, respectively (Fig. 2A). Of note, a recent pharmacokinetic analysis of dasatinib in lung cancer patients revealed that peak concentrations of dasatinib were in the range of 300 ng/mL (615 nM) at the maximum tolerated dose of 140 mg daily, a dose approved for use in leukemias (29). Imatinib was less potent when tested in the same cell lines with respective IC_{50}s of 1.2 and 1.0 μM for the *DDR2* mutant NCI-H2286 and HCC-366 cell lines (Supplementary Fig. S2A). Dasatinib and imatinib were less effective against the A549 cell line, which is known to harbor a *KRAS* mutation and does not have any *DDR2* mutations (calculated IC_{50} of 7.4 μM for dasatinib and 2.3 μM for imatinib). Consistent with previous reports, the NCI-H1703 SCC cell line, which contains a *PDGFRA* amplification, was sensitive to both drugs, serving as a positive control for our assay (30, 31). Notably, no other somatic mutations have been reported in the Catalogue of Somatic Mutations in Cancer (COSMIC) database for NCI-H2286 or HCC-366 lines to suggest alternative dasatinib targets; a previous report examining the drug sensitivities of 83 NSCLC cell lines identified HCC-366 as the lung SCC line most sensitive to dasatinib, although NCI-H2286 and NCI-H1703 were not assayed (32). Treatment of the *DDR2* mutant cell lines with dasatinib appeared to lead to cell death instead of cell-cycle arrest, as measured by trypan blue exclusion (Supplementary Fig. S2B). Dasatinib treatment was associated with an increase in cellular annexin V staining, suggesting that the treated cells died by apoptosis (data not shown).

To validate DDR2 as a relevant target of dasatinib in SCCs, we ectopically expressed a *DDR2* transgene with a threonine-to-methionine mutation at amino acid 654, a mutation site shown previously to render DDR2 insensitive to dasatinib in a manner similar to the ability of the T790M mutation in *EGFR* to confer acquired resistance to the tyrosine kinase inhibitors erlotinib and gefitinib (33). We introduced the dasatinib-insensitive DDR2 "gatekeeper" mutant in *cis* with the observed L239R and I638F mutations in the HCC-366 and NCI-H2286 cell lines, respectively, as well as alone in NCI-H1703. Expression of the gatekeeper mutation led to a decrease in dasatinib sensitivity in both *DDR2* mutant cell lines and had a modest effect on NCI-H1703 (Fig. 2B;

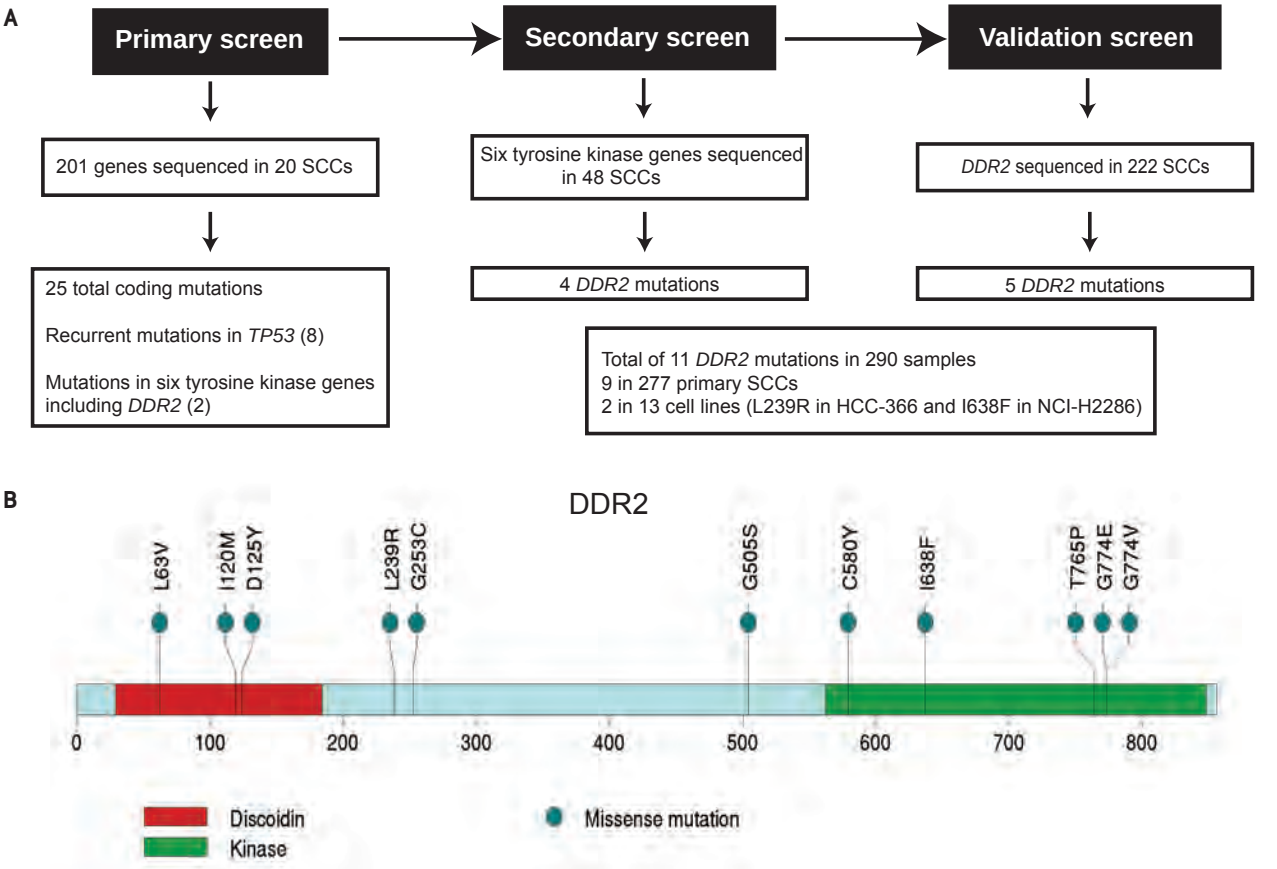

Figure 1. Sequencing of squamous lung cancer samples identifies recurrent mutations in *DDR2*. **A,** primary, secondary, and validation screens for *DDR2* mutations in lung SCC samples. **B,** amino acid sequence of DDR2, with positions of identified mutations shown in the context of the known domain structure of DDR2.

transgene expression is shown in Supplementary Fig. S2E). Whereas the calculated IC_{50} for NCI-H1703 did not change with ectopic expression of the gatekeeper, the IC_{50} increased by 35-fold for NCI-H2286 and 209-fold for HCC-366. Of interest, a parallel sequencing project in our laboratory identified a T654I mutation in DDR2 in a primary endometrial carcinoma sample (data not shown).

Dasatinib was originally designed as an inhibitor of Src and is a multitargeted tyrosine kinase inhibitor (34). Dasatinib treatment is associated with toxicity in patients, including myelosuppression and the development of pleural and pericardial effusions (35, 36). In an attempt to identify additional agents that could potently inhibit DDR2 with less associated toxicity, we screened a panel of 20 tyrosine kinase inhibitors that, on the basis of their respective structures, were predicted to have the potential to inhibit DDR2. We found that nilotinib, a second-generation BCR-Abl inhibitor, as well as AP24534, a third-generation BCR-Abl inhibitor that displays activity against BCR-Abl and imatinib-resistant BCR-Abl (36), inhibited the proliferation of SCC lines harboring *DDR2* mutations (Supplementary Fig. S2C and D). We observed that AP24534 treatment resulted in a greater degree of inhibition than did nilotinib treatment, a finding in agreement with calculated *in vitro* K_d values of 35.4 nM for nilotinib and 9.0 nM for AP24534, compared with 5.4 nM for dasatinib (Supplementary Table S1).

shRNAs Targeting *DDR2* Kill *DDR2* Mutant SCC Cell Lines

As an independent measure of *DDR2* dependency, we expressed shRNAs targeting *DDR2,* using lentiviral vectors in the NCI-H2286, HCC-366, and NCI-H1703 cell lines. We screened a set of shRNA-expressing plasmids for the ability to knock down *DDR2* mRNA expression by real-time PCR in NCI-H2286 cells and selected 2 hairpins for further analysis because of their ability to reduce *DDR2* mRNA levels by approximately 50% (data not shown). We observed that knockdown of DDR2 by these 2 sh-RNAs led to a reduction in proliferation of the 2 *DDR2* mutant cell lines but not of *PDGFRA*-amplified NCI-H1703 cells, which had been sensitive to imatinib and dasatinib in our proliferation assays (Fig. 2C). The reduction in proliferation appeared to correlate with the degree of knockdown because the observed phenotype was greater with shRNA-2 than shRNA-5 (Fig. 2C) and seemed to result from cell death, not cell-cycle arrest (data not shown).

To assess the specificity of the observed knockdown phenotype, we performed a similar experiment in NCI-H2286 and HCC-366 cells ectopically expressing their described

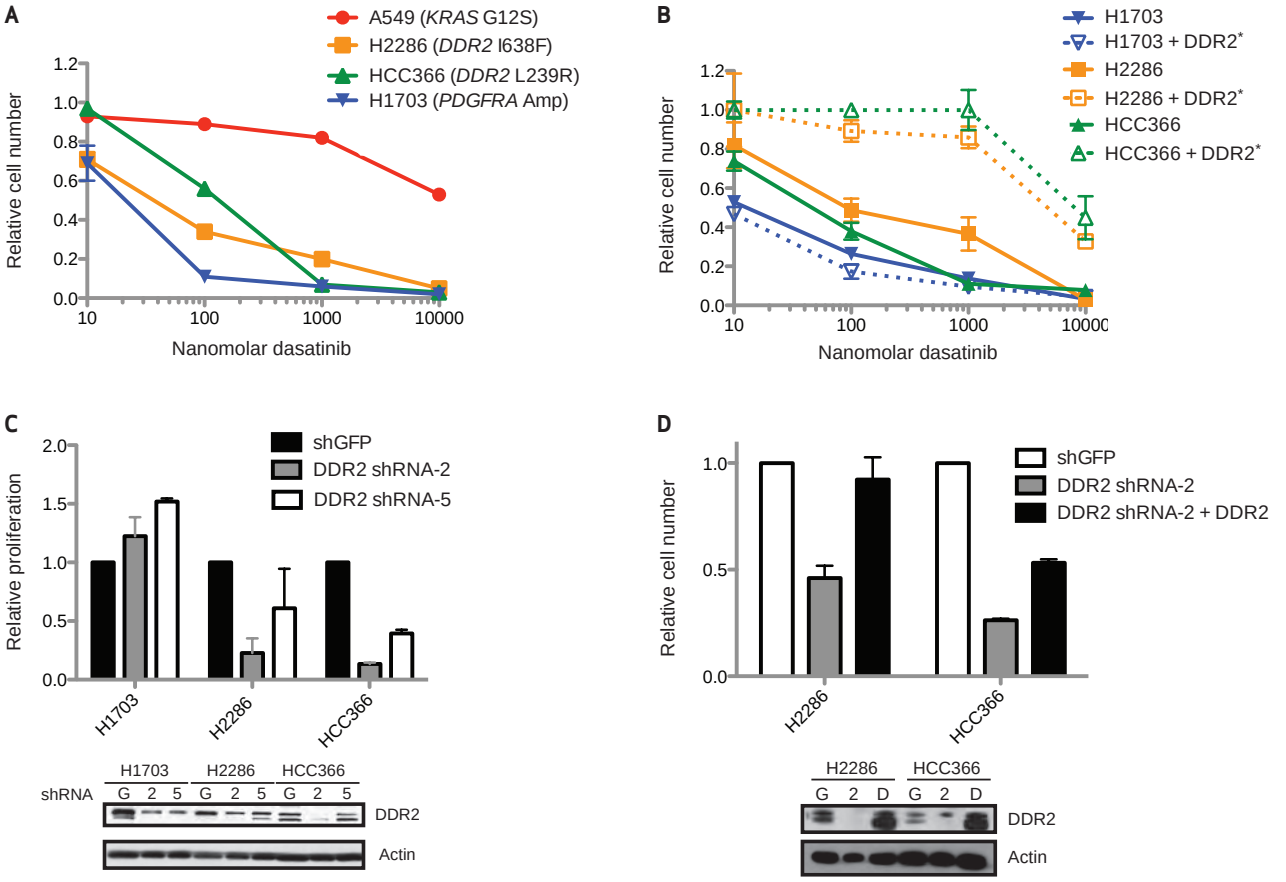

Figure 2. Lung cancer cell lines with *DDR2* mutations are sensitive to drugs and RNA interference targeting DDR2. **A,** proliferation of A549, NCI-H2286, HCC-366, and NCI-H1703 grown for 6 days in the presence of various concentrations of dasatinib. Proliferation shown relative to untreated cells at the same time point. SEs are shown for triplicate samples. **B,** proliferation of NCI-H2286 and HCC-366 cell lines ectopically expressing the T654M gatekeeper mutation in *DDR2*, labeled DDR2*. The 6-day proliferation in the presence of dasatinib is shown in **A.** For NCI-H2286 and HCC-366, the gatekeeper mutation is expressed in *cis* with the DDR2 mutation found in the cell line. **C,** Proliferation measured as in **A** for NCI-H2286, HCC-366, and NCI-H1703 cells stably expressing shRNA vectors targeting either green fluorescent protein (GFP) or the 3′ UTR of *DDR2* (DDR2 shRNA-2) or the coding sequence of *DDR2* (DDR2 shRNA-5). Proliferation is measured after 4 days in culture, compared with day 1. SEs are shown for triplicate samples. Inset, immunoblot showing relative levels of DDR2 in the cell lines used in the experiment. "G," cells expressing shGFP; and "2" and "5," the numbered *DDR2* targeted hairpins. **D,** The 4-day proliferation of *DDR2* mutant NCI-H2286 and HCC-366 cell lines stably expressing ectopic *DDR2* following knockdown of DDR2 by a shRNA targeting the 3′ UTR of *DDR2* (shRNA-2). Proliferation of triplicate samples is presented as in **A,** relative to cells transduced with a shRNA targeting GFP. Protein levels of DDR2 are shown in the immunoblot below. "G," shGFP, "2," expression of shRNA2; and "D," expression of shRNA2 and *DDR2*.

mutated forms of *DDR2* (I638F and L239R, respectively) and then knocked down endogenous DDR2 with shRNA-2, which targets the 3′ untranslated region (UTR) of *DDR2* and would not be expected to interfere with ectopic expression of *DDR2*. We observed for both NCI-H2286 and HCC-366 that ectopic expression of *DDR2* attenuated the antiproliferative effect of endogenous DDR2 knockdown and that the effect was of greater magnitude in NCI-H2286, perhaps owing to a greater degree of off-target effects in HCC-366 (Fig. 2D).

DDR2 Mutations Are Associated with Dasatinib Sensitivity *In vivo*

To analyze the effects of dasatinib treatment in a somewhat more physiologic setting, we performed xenograft studies in athymic nude mice in which we injected cohorts of mice with NCI-H2286, HCC-366, NCI-H1703, and A549 cells. HCC-366 cells did not form tumors in the mice and could not be analyzed further. Following tumor formation of the 3 tested lines, mice were treated with dasatinib at 50 mg/kg by oral gavage for 2 weeks or with vehicle control. Dasatinib treatment led to a decrease in tumor size in the NCI-H1703 and NCI-H2286 lines, but not in A549, consistent with our *in vitro* results (Fig. 3).

DDR2 Mutations Are Oncogenic, and *DDR2*-Driven Transformation Is Dasatinib Sensitive

We examined whether *DDR2* mutations could confer an oncogenic gain-of-function phenotype. Ectopic expression of a subset of the *DDR2* mutants identified in our primary and secondary screens ($n = 2$ of 6) promoted the formation of colonies in soft agar of NIH-3T3 cells (Supplementary Fig. S3A). Colony formation was greatest in the L63V and I638F mutants at a level comparable to that driven by expression of the gain-of-function L858R mutation in *EGFR* and

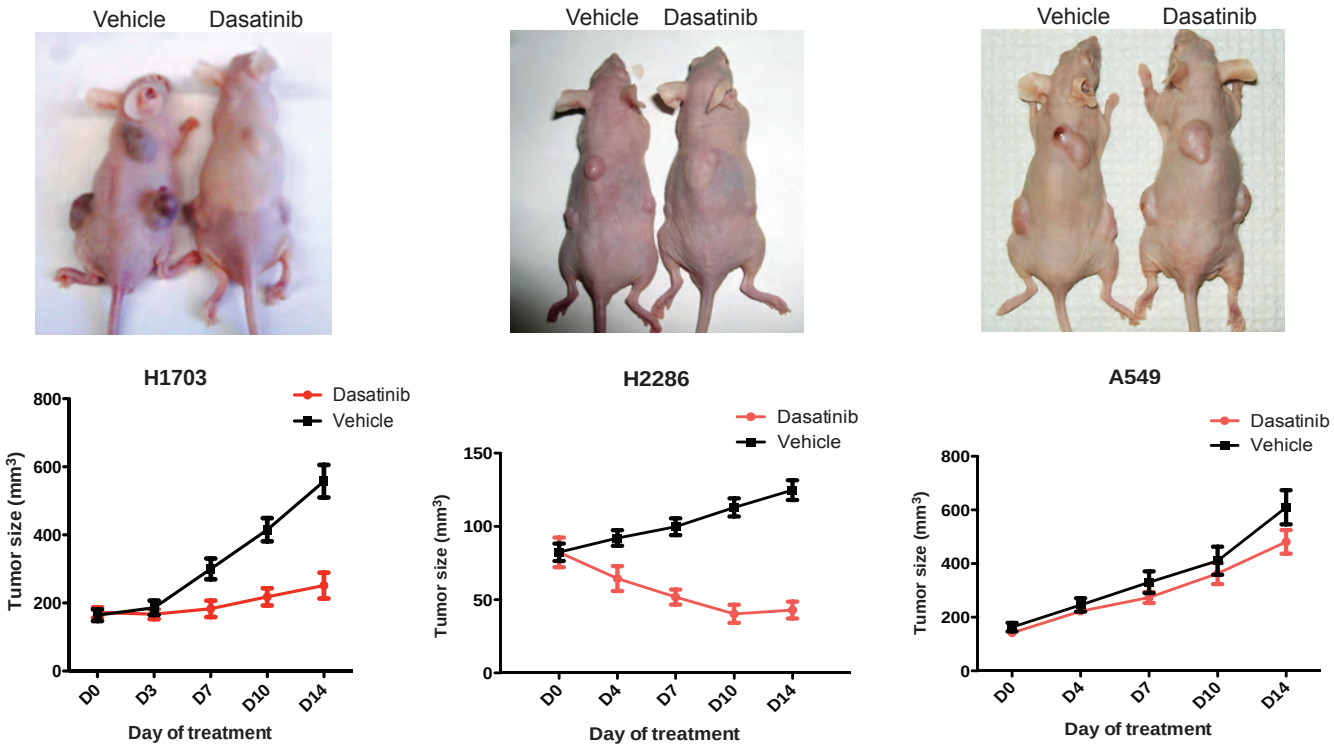

Figure 3. Xenografts of squamous lung cancer cell lines demonstrate antitumor effects of dasatinib *in vivo*. Athymic nu/nu mice were injected s.c. with A549, NCI-H1703, HCC-366, and NCI-H2286 cells ($n = 10$) and treated with dasatinib or vehicle for 2 weeks following tumor formation. Shown are representative images of mice from each cohort, as well as measurements of tumor size. Tumors did not form in the mice injected with HCC-366; these mice could not be analyzed further.

modest in the remainder of the genotypes. Colony formation could be inhibited with a single application of dasatinib at the time of plating in the case of the L63V mutant, the one that reproducibly formed the most colonies in our assay (Fig. 4A). Dasatinib treatment also inhibited the colony formation of NIH-3T3 cells expressing the L858R mutation in *EGFR*, consistent with previous reports, and did so to a lesser extent in NIH-3T3 cells stably expressing the activating G12V *KRAS* mutation (Fig. 4A; refs. 32, 37).

Because the observed gain-of-function phenotype was modest for many of the *DDR2* mutants in NIH-3T3 cells, we evaluated the transforming potential of *DDR2* in the interleukin-3 (IL-3)–dependent hematopoietic cell line Ba/F3. We observed that ectopic expression of all 6 *DDR2* mutants identified in our primary and secondary screens led to IL-3–independent growth of Ba/F3 cells, as did high expression levels of wild-type *DDR2;* no differences were observed in the time to transformation or the rate of IL-3–independent proliferation (Supplementary Fig. S3B). A kinase-dead *DDR2* transgene (K608E) did not support the IL-3–independent growth of Ba/F3 cells (Supplementary Fig. S3B). Although culture with the less potent DDR2 inhibitor imatinib did not lead to significant killing of Ba/F3 cells expressing *DDR2* mutations, compared with cells grown in the presence of IL-3, culture with dasatinib led to cell death in all cell lines expressing *DDR2* mutants, with a mean calculated IC_{50} of 680 nM for the mutants and 30 μM for the control (Fig. 4B; Supplementary Fig. S3C). The third-generation BCR-Abl inhibitor AP24534 was also effective in killing the IL-3–independent Ba/F3 cells expressing mutant forms of *DDR2*, suggesting that this class of drugs may be effective against *DDR2*-driven neoplasms, whereas the second-generation BCR-Abl inhibitor nilotinib demonstrated modest activity against the *DDR2*-transformed Ba/F3 cells (Supplementary Figs. S3D and S4A). Survival of Ba/F3 cells in the absence of IL-3 was associated with maintenance of STAT5 phosphorylation, as has been previously shown (Supplementary Fig. S4B; ref. 38).

DDR2-Transformed Cell Lines Maintain Src Phosphorylation and Are Especially Sensitive to Dual Inhibition of DDR2 and Src

Given that the type I kinase inhibitor dasatinib was more potent in *DDR2*-transformed Ba/F3 cells than the more target-specific type II inhibitors nilotinib and imatinib, we sought to test whether the potency of dasatinib in this system might be due to the effects of dasatinib on kinases in addition to DDR2. DDR2 has previously been shown to require Src for maximal kinase activity (16), and we observed that levels of phosphorylated Src were maintained in Ba/F3 cells expressing *DDR2* mutants in the absence of IL-3 (Supplementary Fig. S4B). To determine whether the ability of *DDR2* mutations to confer IL-3–independent proliferation in Ba/F3 cells might depend on both DDR2 and Src activity, we treated Ba/F3 cells expressing *DDR2* with AZD0530, a highly

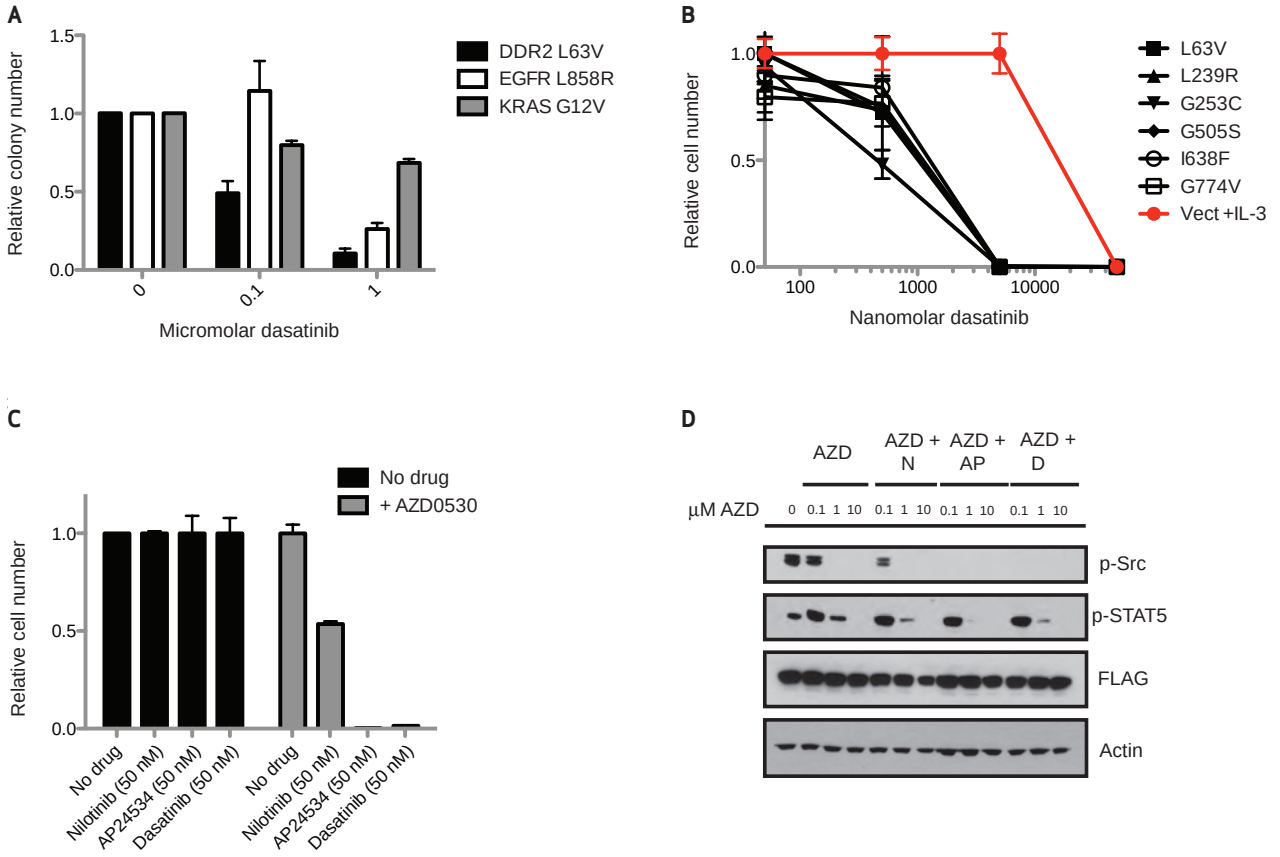

Figure 4. Ectopic expression of *DDR2* mutants leads to cellular transformation that can be blocked by dasatinib or combination tyrosine kinase inhibitor treatment. **A,** results from soft agar assay in which 3T3 fibroblasts expressing the L63V *DDR2* mutation, the L858R *EGFR* mutation, or the *KRAS* G12V mutation were plated in soft agar in the presence of various concentrations of dasatinib. Colony number of 6 independent samples with SEs is shown. **B,** proliferation at 4 days of Ba/F3 cells expressing vector only or 1 of 6 *DDR2* mutations seen in cells grown in the presence of dasatinib. For the vector, control cells are grown in the presence of IL-3 to maintain viability, and for *DDR2* mutants, all cells are IL-3 independent and cultured in the absence of IL-3. Proliferation is shown relative to untreated cells at the same time point for triplicate samples with SEs. **C,** proliferation of Ba/F3 cells expressing *DDR2* L63V cocultured with 50 nM of nilotinib, AP24534, or dasatinib with or without 500 nM AZD0530; proliferation is relative to untreated cells grown in parallel. **D,** immunoblots of *DDR2* L63V-transformed Ba/F3 cells treated for 2 days with the depicted concentrations of AZD0530 (AZD) in addition to 50-nM nilotinib (N), AP24534 (AP), or dasatinib (D). The first lane is an untreated sample. Shown are immunoblots probed with antibodies against phospho-Src Y416, phospho-STAT5 Y694, FLAG-DDR2, and actin.

selective Src-family kinase inhibitor that displays minimal activity against DDR2, in comparison with the other inhibitors described in this article (*in vitro* K_d, 291 nM; Supplementary Table S1; ref. 39). As with nilotinib treatment, AZD0530 had a modest effect on the proliferation of the IL-3–independent *DDR2*-expressing Ba/F3 cells (Supplementary Fig. S4A and C). However, when Ba/F3 cells expressing the L63V *DDR2* mutation were grown in 50-nM nilotinib, a concentration associated with little effect on proliferation of wild-type Ba/F3 cells or Ba/F3 cells expressing *DDR2* mutations (Supplementary Fig. S4A), the addition of AZD0530 led to a marked reduction in proliferation of Ba/F3 cells expressing *DDR2* L63V; this finding suggested that coordinated activity of DDR2 and Src-family kinases may be required for the ability of *DDR2*-mutated Ba/F3 cells to grow in the absence of IL-3, thereby providing a possible explanation for the potency of dasatinib in this system (Fig. 4C; Supplementary S5A). A similar additive effect of AZD0530 was observed when the Ba/F3 cells were cotreated with AZD0530 and 50 nM of either AP24534 or dasatinib (see Fig. 4C for L63V; for a more detailed version of this experiment, including additional *DDR2* mutants, see Supplementary Fig. S5). AZD0530 reduced Src and STAT5 phosphorylation in a dose-dependent fashion in the *DDR2* L63V-expressing Ba/F3 cells when used as a single agent or in combination with nilotinib, AP24534, or dasatinib (Fig. 4D; Supplementary Fig. S4D).

Observation of a *DDR2* Kinase Domain Mutation in a Clinical Trial Subject with a Radiographic Response to Combination Therapy with Dasatinib and Erlotinib

Two recent early-phase clinical trials of dasatinib have been reported in which subjects with advanced-stage lung cancer were treated with either dasatinib or a combination of dasatinib and erlotinib (29, 40). One of 7 subjects with a lung SCC exhibited significant shrinkage in tumor size while undergoing therapy with a combination of dasatinib and erlotinib; unlike the other subject in the study who exhibited

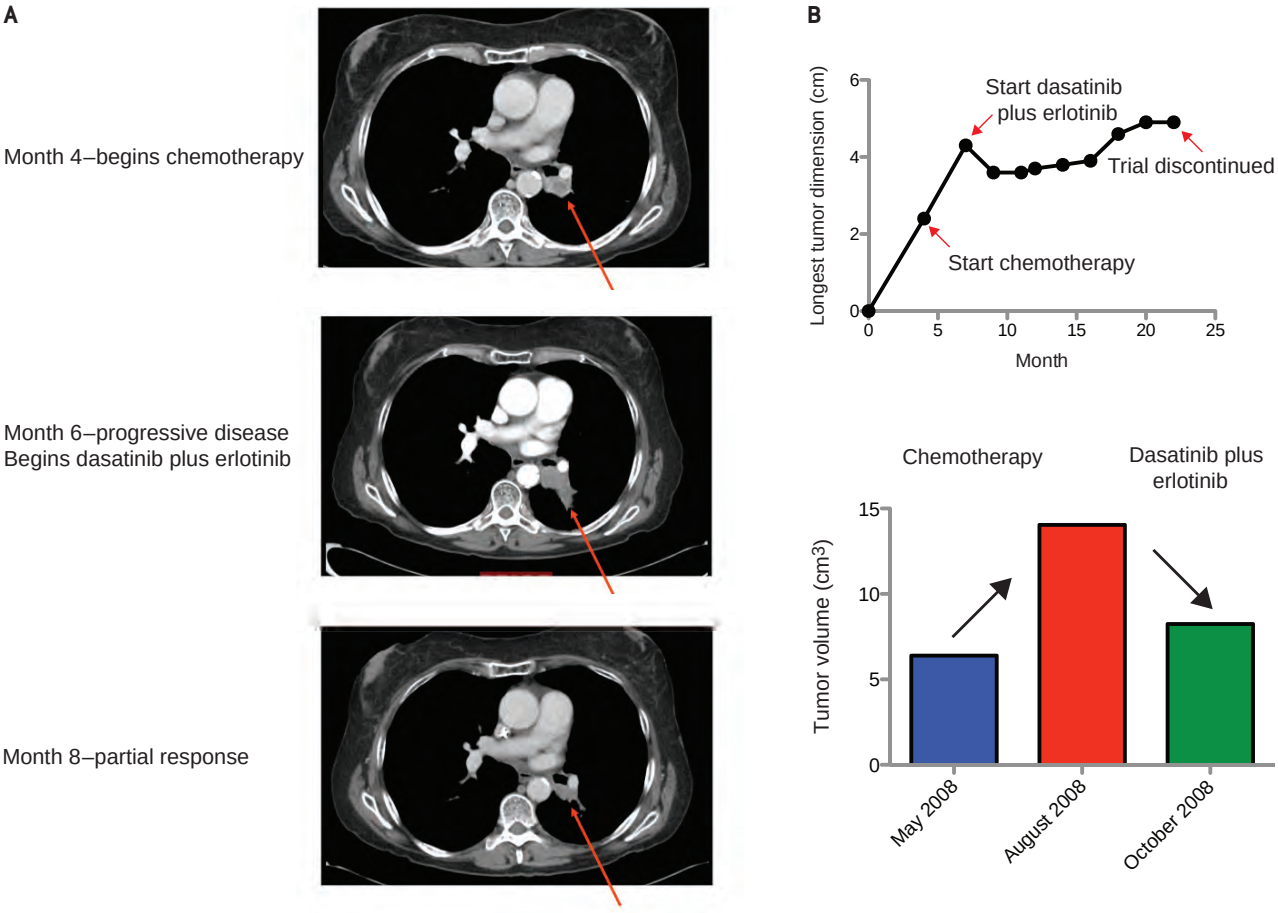

Figure 5. Radiographic response of a patient with a S768R *DDR2* mutation treated with dasatinib plus erlotinib. **A,** computed tomography (CT) images from a lung SCC patient treated with chemotherapy and later with dasatinib plus erlotinib. Serial CT scans are shown at the time of chemotherapy, at the initiation of study treatment with dasatinib and erlotinib, and after 2 months of treatment with dasatinib plus erlotinib. **B,** top, tumor dimension measurements from the subject in **A,** starting 4 months prior to chemotherapy treatment and extending to the time at which combination therapy with dasatinib and erlotinib was discontinued. Bottom, bar graph depicting measured tumor volume according to Response Evaluation Criteria in Solid Tumors prior to chemotherapy, following chemotherapy, and after 2 months of dasatinib plus erlotinib therapy.

a response to treatment, and who had adenocarcinoma, no evidence of *EGFR* mutation was found in the subject with SCC. The patient was a 59-year-old white woman with a one-third pack–per-day smoking history for 38 years who quit 1 year before her diagnosis of lung cancer. She was found to have a left lower lobe stage I (tumor, 2; node, 0; metastasis, 0) SCC and received primary treatment with weekly carboplatin and paclitaxel, with concomitant 70 Gy of radiation, resulting in a complete response. However, approximately 1 year later, the disease progressed within the radiation field, and treatment was initiated with a standard dose of carboplatin and paclitaxel, without response. She then began combination dasatinib and erlotinib therapy on protocol. A restaging computed tomographic (CT) scan after nearly 2 months indicated tumor shrinkage, and the patient experienced improved symptoms (resolved dyspnea and cough; Fig. 5A and B). She remained on the treatment regimen for 14 months until it had to be discontinued due to treatment-induced airspace disease and pleural effusions.

We performed directed sequencing of *DDR2* in a pretreatment tumor specimen derived from this individual and identified a novel *DDR2* kinase domain mutation, S768R, which was present in 844 of 3020 (28%) reads obtained by 454 sequencing (data not shown) and independently verified by Sanger sequencing (Supplementary Fig. S6). The mutation could not be verified as somatic because no normal DNA was available for this individual, who is deceased. This correlation could not be further explored because no other SCC subjects responded to therapy in this study or a subsequent one of dasatinib alone ($n = 13$ total). We performed 3-dimensional modeling of the S768R mutation in the context of the DDR2 kinase domain, which suggested that the S768R substitution is likely to alter the kinase activity of DDR2 (Supplementary Fig. S7).

DISCUSSION

We report the identification of 11 novel mutations in *DDR2* in a screen of 290 SCC samples, yielding an overall mutation rate of 3.8% in all samples and 3.2% in primary SCCs, a rate comparable to the fraction of lung adenocarcinoma patients bearing *ALK* fusions, a genomic event associated with

dramatic responses to the *ALK* inhibitor crizotinib (10, 41). At this time, it remains an important question whether *DDR2* mutations will be primarily found in SCCs of the lung or whether they may be present in SCCs originating from other tissues, such as those of the head and neck, the skin, or tumors of different histologic types. Furthermore, the rate of *DDR2* mutations was lower in our validation screen than in our initial mutation screens, and it will likely take additional sequencing efforts, such as The Cancer Genome Atlas, which is upcoming, to further define the prevalence of *DDR2* mutations. Of note, we have found 2 additional *DDR2* mutations in a sample set of endometrial carcinomas (T654I and T685S), as well as a mutation in a colorectal cancer patient (T692N), supporting the possibility that *DDR2* mutations may be present in multiple cancer types. A search of the COSMIC database is notable for *DDR2* mutations in renal cell carcinoma, glioblastoma multiforme, and the previously mentioned lung adenocarcinoma samples. In addition, our initial and secondary screens consisted largely of samples from the United States whereas the validation screen consisted of more samples from European patients, suggesting that demographics may also affect the rate of observed *DDR2* mutations. A recent report in which >1500 genes were sequenced in a cohort of 63 squamous cell lung cancers, using mismatch repair technology, did not identify any *DDR2* mutations (42); however, we calculate that the sample size was not large enough to detect a statistically significant difference in the rates of *DDR2* mutations, when compared with our study, in which a power of 0.8 and alpha of 0.05 were assumed (43).

We evaluated the effects of ectopic expression of 6 mutant forms of *DDR2* in NIH-3T3 cells and Ba/F3 cells and showed that mutated *DDR2* could function as an oncogene in either context, although with differing potency. We did not complete an assessment of all identified *DDR2* mutants, nor did we evaluate the effects of expression of mutated *DDR2* in the more appropriate context of primary squamous lung cells in a mouse or other model organism. The creation of these models is currently in progress and will be critical to more fully characterize the function of mutated *DDR2*.

The precise mechanism by which mutated *DDR2* promotes cellular transformation is unclear. Whereas ectopic expression of *DDR2* correlated with STAT5 and Src phosphorylation in transformed Ba/F3 cells and chemical inhibition of Src and DDR2 appeared to exhibit an additive, if not synergistic, effect in DDR2-transformed Ba/F3 cells, the mechanism by which mutations in *DDR2* activate downstream signaling is not known. It is possible that the kinase domain mutations, in a manner similar to the modeled mutation at S768, alter the kinase activity of DDR2. The observation that ectopic expression of wild-type DDR2 was sufficient to transform Ba/F3 cells suggests that increased DDR2 signaling activity is a potential mechanism of transformation. It is also possible that mutations in the discoidin domain or unclassified regions of DDR2 could impact upon the ligand binding or localization of DDR2; prior reports have shown that DDR2 mutations in familial spondylometaepiphyseal dysplasia alter the ligand binding and membrane trafficking of DDR2 (44, 45).

In addition to discussing identification of recurrent somatic mutations in the *DDR2* kinase gene, we show that dasatinib can efficiently inhibit the proliferation of *DDR2*-mutated SCC cell lines *in vitro* and *in vivo,* as well as cells ectopically expressing mutant *DDR2*. Together, these data identify a potential first therapeutic target in lung SCC for which clinically approved drugs already exist, thereby providing a rationale for clinical trials of tyrosine kinase inhibitors. We also report a *DDR2* kinase domain mutation in a patient with lung SCC who exhibited a radiographic response to the combination of dasatinib and erlotinib and who did not harbor an *EGFR* mutation. Although this is an interesting result, we think that caution should be exercised in its interpretation, given that only 1 response was reported in a SCC patient in the trial, thus precluding a thorough assessment of the correlation. Further, the subject discontinued therapy in the context of unacceptable toxicity after 14 months of treatment, suggesting that investigation of tyrosine kinase inhibitors other than dasatinib is warranted. Although it is not possible to definitively conclude that this patient responded specifically to dasatinib treatment due to a *DDR2* mutation, we believe it is unlikely that the response was due to erlotinib, in light of the absence of an *EGFR* kinase domain mutation and the previously noted GI_{50} of 990 nM for erlotinib in the *DDR2* mutant HCC-366 cell line (32). A search of the literature did not uncover any earlier reports of erlotinib as a potent inhibitor of DDR2, and our initial experiments in the *DDR2* mutant cell lines HCC-366 and NCI-H2286 indicate a sensitivity to erlotinib that is at least 10-fold less than sensitivity to dasatinib (data not shown).

In conclusion, we hope our data may stimulate the initiation of larger clinical trials of dasatinib or other tyrosine kinase inhibitors in patients with lung SCC and testing of these patients for *DDR2* mutations, potentially leading to a less toxic and more effective treatment for this deadly disease.

METHODS

Ethics Statement

All animal experiments were performed under an Institutional Animal Care and Use Committee–approved animal protocol at the Dana-Farber Cancer Institute (Boston, MA) and all experiments involving human DNA were performed in accordance with Institutional Review Board (IRB)–approved protocols as described below.

Collection of Lung SCC Samples

For the primary and secondary screens, tumor samples were obtained from the Dana-Farber Cancer Institute/Brigham and Women's Hospital/Harvard Cancer Center (Boston, MA) under Institutional Protocol 02-180, approved by the Dana-Farber/Harvard Cancer Center IRB in September 2002 and renewed yearly thereafter. This is a general tissue collection protocol for patients with lung cancer who have consented to tissue collection for research, including DNA sequencing, prior to surgery. All patients with resectable, biopsy-proven lung SCCs (as diagnosed by a board-certified anatomic pathologist) were eligible. These eligible subjects participated in a detailed informed consent procedure prior to enrollment on the protocol, which included a discussion of the use of tissue samples for DNA sequencing studies and written documentation of consent. In addition, DNA samples from de-identified patients with lung SCCs were obtained from the Ontario Cancer Institute for the primary and secondary screens as part of a Dana-Farber/Harvard Cancer

Center IRB-approved collection of de-identified tumor samples for DNA sequencing studies. IRB approval for collection of de-identified samples was subject to a review of the local IRB-approved protocols for all external sites to ensure that an adequate informed consent process had taken place.

The validation screen was performed at the University of Cologne and Max Planck Institute for Neurological Research (Cologne, Germany). Samples were taken in accordance with a tissue collection protocol approved by the University of Cologne Ethics Committee; this protocol included a detailed informed consent process with discussion of genetic testing prior to the subject's surgery and written documentation of consent. Again, all patients with biopsy-proven lung SCCs were eligible regardless of disease stage, as long as their tumors were considered resectable. De-identified samples were also collected from additional European sites, including Haukeland University Hospital (Bergen, Norway), University Hospital Zurich (Zurich, Switzerland), Université Joseph Fourier (Grenoble, France), Oslo University Hospital (Oslo, Norway), Jena University Hospital (Jena, Germany), and University Medical Center Groningen (Groningen, The Netherlands). At all sites, samples were obtained in accordance with an IRB-approved tissue collection protocol, and the collection of de-identified samples from these sites was approved by the University of Cologne Ethics Committee after a review of local collection protocols.

For the single patient sample obtained from the recent clinical trial of combination therapy with dasatinib and erlotinib for advanced lung cancer, DNA was provided to the University of Cologne by the H. Lee Moffitt Cancer Center (Tampa, FL). DNA was obtained under an IRB-approved protocol at the H. Lee Moffitt Cancer Center and the sequencing of the de-identified sample by both the 454 and the Sanger method was performed with the approval of the University of Cologne Ethics Committee after a review of the collection protocol.

In all instances, specimens were continuously selected at the site of surgery, to avoid sampling bias, and all samples were de-identified prior to processing for DDR2 sequencing. When available, de-identified correlative clinical data were provided with the samples, although these data were not available to the investigators before sample genotyping. Patients with a prior history of tumors involving a visceral organ site were excluded to avoid the inclusion of metastases.

DDR2 *Sequencing*

DDR2 was sequenced from genomic DNA obtained from lung SCC cell lines and patient samples by conventional Sanger sequencing. In the discovery set, 20 patient samples and matched normal DNA were used for sequencing 201 genes, including 90 kinases. All mutations were confirmed as somatic. Mutations were identified using an automated mutation caller and then verified manually with comparison made to the matched normal sequence in the case of all primary tumor samples. In the secondary screen, 35 additional patient samples and 13 SCC cell lines were used for sequencing the 6 mutated tyrosine kinases identified in the primary screen (*DDR2, FGFR2, NTRK2, JAK2, CDK8,* and *FLT3)*. In the validation screen, 222 total samples underwent sequencing of the *DDR2* gene. In all cases except D125Y, matched normal DNA was available to verify the mutation as somatic.

Cell Culture

A549, NCI-H2286, HCC-366, and NCI-H1703 cells were obtained from the core collection at the Dana-Farber Cancer Institute, having previously been purchased from the American Type Culture Collection and used to establish a collection of early-passage lung cancer cell lines that were analyzed by fingerprinting and single-nucleotide polymorphism arrays (32). All cells used for the experiments described in this article were obtained from freezes made at that time, and no further validation was performed. Lung cancer cell lines were grown in RPMI 1640 (Invitrogen) with 10% fetal calf serum, NIH-3T3 cells were grown in Dulbecco's modified Eagle's medium (Mediatech) with 10% bovine serum, and Ba/F3 cells were grown in RPMI supplemented with 10% bovine serum and IL-3 (BD Biosciences) at 10 ng/mL. For IL-3 withdrawal experiments, Ba/F3 cells were collected via centrifugation, washed once in sterile PBS, and then resuspended in media without IL-3. Colony formation assays in NIH-3T3 cells were performed in 6-well plates in which 25,000 NIH-3T3 cells were plated in triplicate in 1 mL of 0.33% top agar over 2 mL of 0.5% bottom agar. After 3 weeks, colonies were counted using NIH ImageJ software.

Vectors

The full-length DDR2 cDNA was obtained from Origene and cloned into the EcoRI site of the retroviral vector pWzlBlast and pBabepuro following the addition of a C-terminal FLAG tag by PCR. Mutants were generated by site-directed mutagenesis using the QuikChange Site-Directed Mutagenesis Kit (Strategene). All mutations were verified by sequencing. shRNA lentiviral vectors for DDR2 were obtained from The RNAi Consortium at the Broad Institute (Cambridge, MA; 46, 47). DDR2 shRNA-2 corresponds to TRC clone TRCN0000121117, with hairpin sequence 5′-CCGG-CCCATGCCTATGCCACTCCAT-CTCGAG-ATGGAGTGGCATAGGCATGGG-TTTTTG-3′ targeting the 3′ UTR of DDR2. DDR2 shRNA-5 corresponds to TRC clone TRCN0000121121, with hairpin sequence 5′-CCGG-CCCTGGAGGTTCCATCATTTA-CTCGAG-TAAATGATGGAACCTCCAGGG-TTTTTG-3′ targeting the coding sequence of DDR2. Both hairpins were provided in the pLKO vector. A hairpin targeting GFP (shGFP) was obtained from TRC as well and used as a control.

Viral Infections

The *DDR2* transgene was expressed in the lung cancer cell lines NIH-3T3 and Ba/F3, using retroviral transduction with the pWzl vector, as has been previously described. Briefly, 293T cells were used to generate the virus and were cotransfected with the appropriate pWzl or pBabe vector and a packaging vector using Fugene (Roche). Cells were subjected to 2 rounds of overnight infection in the presence of polybrene. Stable cells were generated using blasticidin selection at 10 μg/mL for 3T3, Ba/F3, and A549; 2 μg/mL for NCI-H2286 and NCI-H1703; and 1 μg/mL for HCC-366. Lentiviral infections were performed per the online TRC protocol (48), with 293T cells transfected with the suggested 3-vector combination of pLKO, VSVG, and delta 8.9. Virus was collected and used to infect the lung cancer cell lines for 6 hours in the presence of polybrene. Stable cell lines were generated using puromycin selection at a concentration of 2 μg/mL for NCI-H2286 and 4 μg/mL for NCI-H1703, A549, and HCC-366.

Cell Proliferation and Viability Assays

Cell proliferation was measured with the Cell-Titer-Glo Reagent (Promega) per the manufacturer's instructions. For experiments with the SCC cell lines, cells were plated in clear-bottomed 96-well plates at a density of 1,500 cells per well. The following day the drug was added, and cell proliferation for the SCC lines was measured 6 days later. For Ba/F3, cells were plated at 5,000 cells per well and the drug was added the same day. Proliferation was measured 4 days later. Proliferation measurements were made using a standard 96-well plate luminometer/plate reader. Data are shown as relative values in which the luminescence at a given drug concentration is compared with that of untreated cells of the same type. Kinase inhibitors were purchased from LC Laboratories or were synthesized in the laboratory of N.S. Gray at Harvard Medical School. *In vitro* IC_{50}s for DDR2 were determined for all compounds by LanthaScreen time-resolved–FRET kinase activity assays performed by Invitrogen. Cell viability

was measured using a Vi-CELL reader to stain cells with trypan blue and to generate 50 independent images for each measured sample. Annexin V (BD Biosciences) analysis was performed on dasatinib-treated cells 48 hours after the addition of the drug per the manufacturer's protocol. For shRNA experiments, cells were plated at a density of 1,500 cells per well in 96-well plates following puromycin selection. Proliferation was measured 4 days later, in comparison with cells expressing a hairpin targeting GFP.

Immunoblots

Immunoblots were performed using the Nupage System (Invitrogen) per the manufacturer's protocol. Cells were lysed in 1% Nonidet P-40 with protease (Roche) and phosphatase inhibitors (Calbiochem), and protein concentration was assayed with the Bradford reagent (Bio-Rad). Primary antibodies used were Flag-M2 (Sigma), phospho-Y417-Src (Cell Signaling Technologies), phospho-Y694-STAT5 (Cell Signaling), and Actin (Santa Cruz Biotechnology). A DDR2 antibody was generated for this project by Bethyl Laboratories. Secondary horseradish peroxidase–conjugated antibodies were all obtained from Pierce, and proteins were detected by pico-ECL (Thermo Scientific). Images were imported into Adobe Illustrator, using an Epson 4490 scanner. In some cases, brightness and contrast of the scanned images were adjusted for clarity and blots were cropped to display the area of interest in the images. In all cases, adjustment of brightness or contrast was applied uniformly to the image as a whole.

Xenografts

All animal experiments were performed according to institutional guidelines regarding animal safety. Nude mice were injected with the lung cancer cell lines at a density of 2.5 (A549), 3.0 (NCI-H1703), or 5.0 (NCI-H2286 and HCC-366) million cells per injection, in an attempt to control for the variable rates of tumor growth in the animals. Cohorts of 10 mice were injected at 3 sites for each cell type, and the mice were observed until the tumor volume approached 150 mL^3 for A549 and NCI-H1703 or 100 mL^3 for NCI-H1703. At that time, mice were treated with dasatinib at 50 mg/kg or vehicle control daily for 2 weeks, and tumor size was measured during the treatment period.

Statistics

For proliferation and colony formation assays, mean values from a minimum of triplicate samples are reported, as well as SEs calculated by Microsoft Excel. IC_{50} values were obtained using GraphPad Prism software. Power and sample size calculations were performed using the Interactive Statistics Web Resource (43).

Disclosure of Potential Conflicts of Interest

M. Meyerson is a consultant to Novartis; receives research support from Novartis and Genentech; is a founding advisor and consultant to, and an equity holder in, Foundation Medicine; and is a patent holder for EGFR mutation testing, licensed to Genzyme Genetics. N.S. Gray receives research support from Novartis. E.B. Haura was the principal investigator of an industry-sponsored clinical trial of dasatinib and erlotinib in lung cancer, funded in part by Bristol-Myers Squibb and the American Society for Clinical Oncology. R.K. Thomas reports consulting and lecture fees (Sequenom, Sanofi-Aventis, Merck, Roche, Infinity, Boehringer, Astra-Zeneca, Johnson & Johnson, and Atlas-Biolabs) and research support (Novartis, AstraZeneca).

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REFERENCES

1. Lennes IT, Lynch TJ. Quality indicators in cancer care: development and implementation for improved health outcomes in non-small-cell lung cancer. Clin Lung Cancer 2009;10:341–6.
2. West H, Harpole D, Travis W. Histologic considerations for individualized systemic therapy approaches for the management of non-small cell lung cancer. Chest 2009;136:1112–8.
3. Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2005;2:e73.
4. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497–500.
5. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129–39.
6. Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba II, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst 2005;97:339–46.
7. Sasaki T, Rodig SJ, Chirieac LR, Janne PA. The biology and treatment of EML4-ALK non-small cell lung cancer. Eur J Cancer 2010;46:1773–80.
8. Hammerman PS, Janne PA, Johnson BE. Resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. Clin Cancer Res 2009;15:7502–9.
9. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947–57.
10. Shaw AT, Yeap BY, Mino-Kenudson M, Digumarthy SR, Costa DB, Heist RS, et al. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. J Clin Oncol 2009;27:4247–53.
11. Weiss J, Sos ML, Seidel D, Peifer M, Zander T, Heuckmann JM, et al. Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. Sci Transl Med 2010;2:62ra93.
12. Vogel W, Gish GD, Alves F, Pawson T. The discoidin domain receptor tyrosine kinases are activated by collagen. Mol Cell 1997;1:13–23.
13. Vogel W. Discoidin domain receptors: structural relations and functional implications. FASEB J 1999;13 Suppl:S77–82.

14. Shrivastava A, Radziejewski C, Campbell E, Kovac L, McGlynn M, Ryan TE, et al. An orphan receptor tyrosine kinase family whose members serve as nonintegrin collagen receptors. Mol Cell 1997;1:25–34.
15. Leitinger B. Molecular analysis of collagen binding by the human discoidin domain receptors, DDR1 and DDR2. Identification of collagen binding sites in DDR2. J Biol Chem 2003;278:16761–9.
16. Ikeda K, Wang LH, Torres R, Zhao H, Olaso E, Eng FJ, et al. Discoidin domain receptor 2 interacts with Src and Shc following its activation by type I collagen. J Biol Chem 2002;277:19206–12.
17. Olaso E, Labrador JP,Wang L, Ikeda K, Eng FJ, Klein R, et al. Discoidin domain receptor 2 regulates fibroblast proliferation and migration through the extracellular matrix in association with transcriptional activation of matrix metalloproteinase-2. J Biol Chem 2002;277:3606–13.
18. Labrador JP, Azcoitia V, Tuckermann J, Lin C, Olaso E, Manes S, et al. The collagen receptor DDR2 regulates proliferation and its elimination leads to dwarfism. EMBO Rep 2001;2:446–52.
19. Ford CE, Lau SK, Zhu CQ, Andersson T, Tsao MS, Vogel WF. Expression and mutation analysis of the discoidin domain receptors 1 and 2 in non-small cell lung carcinoma. Br J Cancer 2007;96:808–14.
20. Davies H, Hunter C, Smith R, Stephens P, Greenman C, Bignell G, et al. Somatic mutations of the protein kinase gene family in human lung cancer. Cancer Res 2005;65:7591–5.
21. Day E, Waters B, Spiegel K, Alnadaf T, Manley PW, Buchdunger E, et al. Inhibition of collagen-induced discoidin domain receptor 1 and 2 activation by imatinib, nilotinib and dasatinib. Eur J Pharmacol 2008;599:44–53.
22. Raponi M, Zhang Y, Yu J, Chen G, Lee G, Taylor JM, et al. Gene expression signatures for predicting prognosis of squamous cell and adenocarcinomas of the lung. Cancer Res 2006;66:7466–72.
23. Bass AJ, Watanabe H, Mermel CH, Yu S, Perner S, Verhaak RG, et al. SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. Nat Genet 2009;41:1238–42.
24. Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, et al. The landscape of somatic copy-number alteration across human cancers. Nature 2010;463:899–905.
25. Stegmeier F, Warmuth M, Sellers WR, Dorsch M. Targeted cancer therapies in the twenty-first century: lessons from imatinib. Clin Pharmacol Ther 2010;87:543–52.
26. Manley PW, Drueckes P, Fendrich G, Furet P, Liebetanz J, Martiny-Baron G, et al. Extended kinase profile and properties of the protein kinase inhibitor nilotinib. Biochim Biophys Acta 2010;1804:445–53.
27. Li J, Rix U, Fang B, Bai Y, Edwards A, Colinge J, et al. A chemical and phosphoproteomic characterization of dasatinib action in lung cancer. Nat Chem Biol 2010;6:291–9.
28. Karaman MW, Herrgard S, Treiber DK, Gallant P, Atteridge CE, Campbell BT, et al. A quantitative analysis of kinase inhibitor selectivity. Nat Biotechnol 2008;26:127–32.
29. Haura EB, Tanvetyanon T, Chiappori A, Williams C, Simon G, Antonia S, et al. Phase I/II study of the Src inhibitor dasatinib in combination with erlotinib in advanced non-small-cell lung cancer. J Clin Oncol 2010;28:1387–94.
30. Tonon G, Wong KK, Maulik G, Brennan C, Feng B, Zhang Y, et al. High-resolution genomic profiles of human lung cancer. Proc Natl Acad Sci U S A 2005;102:9625–30.
31. Ramos AH, Dutt A, Mermel C, Perner S, Cho J, Lafargue CJ, et al. Amplification of chromosomal segment 4q12 in non-small cell lung cancer. Cancer Biol Ther 2009;8:2042–50.
32. Sos ML, Michel K, Zander T, Weiss J, Frommolt P, Peifer M, et al. Predicting drug susceptibility of non-small cell lung cancers based on genetic lesions. J Clin Invest 2009;119:1727–40.
33. Du J, Bernasconi P, Clauser KR, Mani DR, Finn SP, Beroukhim R, et al. Bead-based profiling of tyrosine kinase phosphorylation identifies SRC as a potential target for glioblastoma therapy. Nat Biotechnol 2009;27:77–83.
34. Quintas-Cardama A, Kantarjian H, Cortes J. Imatinib and beyond—exploring the full potential of targeted therapy for CML. Nat Rev Clin Oncol 2009;6:535–43.
35. Kim LC, Rix U, Haura EB. Dasatinib in solid tumors. Expert Opin Investig Drugs 2010;19:415–25.
36. O'Hare T, Shakespeare WC, Zhu X, Eide CA, Rivera VM, Wang F, et al. AP24534, a pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. Cancer Cell 2009;16:401–12.
37. Song L, Morris M, Bagui T, Lee FY, Jove R, Haura EB. Dasatinib (BMS-354825) selectively induces apoptosis in lung cancer cells dependent on epidermal growth factor receptor signaling for survival. Cancer Res 2006;66:5542–8.
38. Nosaka T, Kawashima T, Misawa K, Ikuta K, Mui AL, Kitamura T. STAT5 as a molecular regulator of proliferation, differentiation and apoptosis in hematopoietic cells. EMBO J 1999;18:4754–65.
39. Hennequin LF, Ballard P, Boyle FT, Delouvrie B, Ellston RP, Halsall CT, et al. Novel 4-anilinoquinazolines with C-6 carbon-linked side chains: synthesis and structure-activity relationship of a series of potent, orally active, EGF receptor tyrosine kinase inhibitors. Bioorg Med Chem Lett 2006;16:2672–6.
40. Johnson FM, Bekele BN, Feng L, Wistuba I, Tang XM, Tran HT, et al. Phase II study of dasatinib in patients with advanced non-small-cell lung cancer. J Clin Oncol 2010;28:4609–15.
41. McDermott U, Iafrate AJ, Gray NS, Shioda T, Classon M, Maheswaran S, et al. Genomic alterations of anaplastic lymphoma kinase may sensitize tumors to anaplastic lymphoma kinase inhibitors. Cancer Res 2008;68:3389–95.
42. Kan Z, Jaiswal BS, Stinson J, Janakiraman V, Bhatt D, Stern HM, et al. Diverse somatic mutation patterns and pathway alterations in human cancers. Nature 2010;466:869–73.
43. Statpages.org [homepage on the Internet]. [updated 2011 February 17; cited 2011 January 12]. Available from: http://statpages.org/proppowr.html
44. Bargal R, Cormier-Daire V, Ben-Neriah Z, Le Mer, rer M, Sosna J, Melki J, et al. Mutations in DDR2 gene cause SMED with short limbs and abnormal calcifications. Am J Hum Genet 2009; 84:80–4.
45. Ali BR, Xu H, Akawi NA, John A, Karuvantevida NS, Langer R, et al. Trafficking defects and loss of ligand binding are the underlying causes of all reported DDR2 missense mutations found in SMED-SL patients. Hum Mol Genet 2010;19:2239–50.
46. Moffat J, Grueneberg DA, Yang X, Kim SY, Kloepfer AM, Hinkle G, et al. A lentiviral RNAi library for human and mouse genes applied to an arrayed viral high-content screen. Cell 2006;124:1283–98.
47. Luo B, Cheung HW, Subramanian A, Sharifnia T, Okamoto M, Yang X, et al. Highly parallel identification of essential genes in cancer cells. Proc Natl Acad Sci U S A 2008;105:20380–5.
48. Broad Institute.org [homepage on the Internet]. 2010 [cited 2011 January 12]. Available from: http://www.broadinstitute.org/rnai/public /resources/protocols